FINAL REPORT FOR CLOSE OUT

Title: Differentiation and Tropisms in Space-Grown Moss

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This grant supported a Space Shuttle experiment on the effects of microgravity on moss cells. Moss provides a rich system for gravitational and spaceflight research. The early phase of the moss life cycle consists of chains of cells that only grow only at their tips. In the moss *Ceratodon purpureus* these filaments (protonemata) grow away from gravity in the dark, in a process called gravitropism. The tipmost cells, the apical cells, contain heavy starch-filled bodies called amyloplasts that probably function in g-sensing and that sediment within the apical cell.

The SPM-A (Space Moss aka SPAM) experiment flew in November - December, 1997 on STS-87 as part of the Collaborative US Ukrainian Experiment (CUE). The experiment was accomodated in hardware purpose-built by NASA KSC and Bionetics and included Petri Dish Fixation Units (PDFU) and BRIC-LEDs. Together, this hardware allowed for the culture of the moss on agar in commercial petri dishes, for unilateral illumination with red light of varying intensity, and for chemical fixation *in situ.*

The key findings of the spaceflight were quite unexpected. Neither the orientation of tip-growth nor the distribution of amyloplasts was random in microgravity.

1. Dark-grown cultures grow in clockwise spirals in microgravity

One major goal of SPM-A was to determine whether the growth of these cells was random in microgravity. Since the cells grow straight up in the dark at 1-g, one prediction for flight was that orientation would be random in space. However, older cultures showed clear clockwise spirals in STS-87. A similar result was found in cultures rotated on a clinostat on earth.

As far as we are aware, the SPM-A results represent only the second time that any gravitropic plant cell or organ grew non-randomly in space (cress roots form arcs related to the seed that produces them). The presence of coordinated clockwise spiral growth in microgravity suggests that there is an endogenous growth polarity in *Ceratodon* that normally is suppressed by gravitropism. A working hypothesis is that the spirals represent a residual spacing mechanism for controlling colony growth and the distribution of branches under some conditions and in some mosses.

2. Amyloplasts that sediment at 1-g were distributed non-randomly in microgravity

Since amyloplasts sediment along the length of apical cells, we predicted that the

amyloplasts would be randomly distributed in microgravity. Instead, dark-grown cells had amyloplasts that were concentrated in clusters towards the tip of the cell meaning that they were distributed non-randomly. This distribution is similar, but not identical to cells that have been inverted or rotated on a clinostat at 1-g.

The finding that amyloplasts in microgravity-grown cells are distributed close to the apex suggests that endogenous forces act constitutively on these organelles. Gravity can counteract these forces as evidenced by amyloplast sedimentation in upright cells on the ground. We have previously shown (Cell Motility and Cytoskeleton 29: 366-74) that sedimentation at 1-g is normally restricted by proteinaceous cables called microtubules. Because microtubules are load-bearing for amyloplasts at 1-g, it is logical that they might also be important for the positioning seen in microgravity.

These data support the idea that both intrinsic and extrinsic forces control the position of dense organelles. These results are also consistent with the hypothesis that the cytoskeleton evolved, at least in part, to prevent the stratification of organelles only by density.

3. Phototropism in microgravity using higher intensities red light

We attempted to separate out gravitropism and phototropism by exposing cells to light of different intensities in space. A clear phototropic response occurred at low intensity. This shows that gravitropism and phototropism compete with each at 1 *g*.

Property None

New Technology or Patents None

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